AMENDMENTS TO THE SPECIFICATION

Please replace the paragraph at page 1, line 4 with the following paragraph:

This application is a continuation U.S. Patent No. 6,333,170, issued December 25, 2001, which is a continuation-in-part ~~of U.S. Patent application 08/440,421 filed May 15, 1995,~~ U.S. Patent No. 5,753,446, issued on May 19, 1998, which is a ~~CIP~~ continuation-in-part of U.S. ~~Patent application 08/323,460 filed October 14, 1994~~ U.S. Patent No. 5,753,446, issued May 19, 1998, which is a continuation-in-part of U.S. Patent No. 5,854,043, issued on December 29, 1998, ~~both of~~ which ~~applications are~~ is a continuations-in-part of U.S.S.N. 08/049,254, filed April 15, 1993, ~~and now~~ which is a divisional application of U.S. Patent No. 5,405,941, issued on April 11, 1995, ~~issued~~. ~~The present application is also a continuation-in-part of U.S.S.N. 08/410,602, filed March 24,1995, and of U.S.S.N 08/472,934, filed June 6, 1995~~. The above-referenced patents and patent applications are incorporated herein by this reference in their entirety.

Please replace the paragraph beginning at page 12, line 37 with the following paragraph:

Certain of the MEKK proteins have been shown to be activated, e.g., as kinases, in response to growth factors and cytokines (such as TNFα and chemoattractants like FMLP and IL-8) and other environmental cues, including stress, as well as expression of activated Ras or other members of the Ras Superfamily, including Rac and Cdc 42. It is demonstrated below that the kinase domain of at least MEKK1 binds to activated Ras in a GTP-dependent manner, implicating that interaction as a potential therapeutic target. Moreover, a Ras effector domain peptide blocks the binding of the MEKK catalytic domain with the GTP-bound form of Ras. In addition, it is shown in the appended Examples that MEKK4 binds to Rac, a low molecular weight GTP binding protein of the Ras superfamily. The sequence of MEKK4 which binds to Cdc42 and Rac has been identified. This sequence IIGQVCDTPKSYDNVMHVGLR (SEQ ID NO:15) occurs around residue 1306-1326 of MEKK4.2 or 599-619 of MEKK4 and peptides from this region can be used to block the binding of the MEKK catalytic domain with Cdc42 and Rac.

Please replace the paragraph beginning at page 45, line 13, with the following paragraph:

In an exemplary embodiment the Ras effector domain or MEKK4 or MEKK4.2 sequence IIGQVCDTPKSYDNVMHVGLR (SEQ ID NO:15) is used to inhibit the interaction of a MEKK protein with a MEKK binding protein.

Please replace the paragraph beginning at page 49, line 24, with the following paragraph:

The portion of MEKK1, for example, which binds to Ras has been identified. The binding of MEKK1 and Ras occurs via the COOH kinase catalytic domain of MEKK1 and residues 17-42 of Ras as determined by the ability of a Ras effector peptide to block the interaction. In addition, the binding of MEKK4.1 and MEKK4.2 to Rac has been localized to the amino acid sequence IIGQVCDTPKSYDNVMHVGLR (SEQ ID NO:15)as described in the appended Examples. Interestingly this sequence has some homology to the Cdc42/Rac interactive binding (CRIB) region. The consensus CRIB sequence, ISXPXXFXHXXHVG (SEQ ID NO:16), even with slight variation within this core sequence, confers binding to Cdc42 and/or Rac GTPases (Burbelo et al. (1995) J. Biol Chem 270:29071-29074). Others have posutlated that Rac1 is an intermediate between Ha-Ras and MEKK in the signaling cascade leading from growth factor receptors and v-Src to JNK activation based on experiments with dominant interfering alleles (Minden et al. (1995) Cell. 81:1147-1157).

Please replace the paragraph beginning at page 45, line 13, with the following paragraph:

One example of a therapeutic compound of the present invention is the nucleic acid encoding the amino acid residues 1306-1326 of MEKK4.2 or 599-619 of MEKK 4. In other embodiments the peptide or fragments thereof can be used. The Cdc42/Rac binding region of a MEKK peptide (IIGQVCDTPKSYDNVMHVGLR (SEQ ID NO:15)) or the nucleic acid which encodes it can be used to inhibit the binding of MEKK and a member of the Ras superfamily. Alternatively, the domain of Rac or Cdc42 to which it binds could be used. In another embodiment the region of the Ras effector domain which blocks the binding of the MEKK catalytic domain with the GTP-bound form of Ras could be used. Alternatively, the portion of the MEKK catalytic domain which binds to Ras could be used to block MEKK-Ras interaction.

Please replace the paragraph beginning at page 78, line 14, with the following paragraph:

Cloning of MEKK 2 and 3. The degenerate primers GA(A/G)(C/T)TIATGGCIGTI AA(A/G)CA (sense; SEQ ID NO:17) and TTIGCICC(T/C)TTIAT(A/G)TCIC(G/T)(A/G)TG (antisense; SEQ ID NO:18) were used in a PCR using first strand cDNA generated from polyadenylated RNA prepared from NIH 3T3 cells. The PCR reaction involved 30 cycles (1 min, 94°C; 2 min, 52°C; 3 min 72°C). A band of approximately 300 base pairs was recovered from the PCR mixture, and the products were cloned into pGEM-T (Promega). The PCR cDNA products were sequenced and compared to the MEKK1 sequence. A unique cDNA sequence of 322 base pairs having significant homology to MEKK1 cDNA was identified and used to screen an oligo (dT)-primed mouse brain cDNA library (Stratagene). The λ phage library was plated and DNA from plaques was transferred to Hybond N filters (Amersham) followed by UV-cross-linking of DNA to the filters. Filters were prehybridized for 2 h and then hybridized overnight in 0.5M $Na_2H_2PO_4$ (pH 7.2), 10% bovine serum albumin, 1 mM EDTA, 7% SDS at 68°C. Filters were washed twice at 42°C with 2 x SSC, once with 1X SSC, and once with 0.5x SSC containing 0.1% SDS. Positive hybridizing clones were purified and sequenced. To resolve GC-rich regions, cDNAs were subcloned into M13 vectors (New England Biolabs), and single strand DNA was sequenced. In all cases, both strands of DNA were sequenced. MEKK 2 encodes a 619-amino aid protein having a mass of 69.7 kDa. MEKK 3 encodes a 626-amino acid protein having a mass of 71 kDa. The two proteins share a common structure with the kinase catalytic domain encoded in the COOH-terminal moiety. The amino-terminal moiety does not encode any definable domain such as a SH2 or SH3 domain sequence.

Please replace the paragraphs beginning at page 79, line 20, with the following paragraph:

Plasmid Expression of MEKK2 and 3. The proteins for MEKK2 and 3 were epitope-tagged at their NH_2 terminus with the hemagglutinin (HA) tag sequence GYPYDVPDYAS (SEQ ID NO:19) using a PCR strategy. For inserting the NH2-terminal epitope tag in MEKK2 and 3, sense oligonucleotides were synthesized having a methionine codon (ATG), 33 bases coding for the GYPYDVPDYAS (SEQ ID NO:19) epitope tag sequences, and 20 bases of MEKK 2 or 3 sequence starting at codon 2. For MEKK2, the sense oligonucleotide was ATGGGGTACCCGTACGACGTGCCGGACTACGCTTCCGATGATCAGCAAGCTTTGAA (SEQ ID NO:20). the sense oligonucleotide for MEKK3 was ATGGGGTACCCGTACGAC GTGCCGGACTACGCTTCCGATGAACAAGAGGCATTAGA (SEQ ID NO:21). The antisnese oligonucleotides for MEKK2 and 3 were AGACTTAGATCTCAGGTCTTC (SEQ ID

NO: 22) encoding a BglII site for MEKK2 and GATTCTGACGTCACTCTGCCT (SEQ ID NO:23) encoding an ActII site for MEKK3. The PCR reactions were performed for 30 cycles using MEKK2 or MEKK3 cDNAs as template. The PCR products were purified, and a second PCR reaction was performed using the first PCR product as template, the MEKK2 or 3 antisense oligonucleotide described above and the common sense oligonucleotide encoding a XbaI restriction site, a consensus Kozak initiation site and 17 bases overlapping with the initiation methionine and HA tag sequence (TCACGTTCTAGAGCCACCATGGGGTACCCGTACGA; SEQ ID NO:24). The resulting PCR products were digested with XbaI and BglII for MEKK2 and XbaI and AatII for MEKK3 and ligated in frame into the appropriate MEKK2 or 3 cDNA. The sequences were confirmed by DNA sequencing and the cDNAs were inserted into the expression plasmid pCMV5. HEK 293 cells were transfected with pCMV5 expression plasmids using Lipofect AMINE (Life Technologies, Inc.) and assayed 48h later. The 12CA5 monoclonal antibody (Berkely Antibody Co.) was used for recognition of the HA epitope tag encoded in expressed MEKK2 and 3.

Antibody Production. Peptides corresponding to COOH-terminal sequences of MEKK3 (CEARQRPSAEELLTHHFAQ; SEQ ID NO:25) and p38 (CFVFPPLDQEEMES; SEQ ID NO:26) were conjugated to keyhole limpet hemocyanin and used to immunize rabbits. Antisera were characterized for specificity by immunoblotting of lysates prepared from appropriately transfected HEK923 cells.

Please replace the paragraph beginning at page 84, line 20, with the following paragraph:

Soluble cell lysates from COS cells transiently transfected with MEKK, mock-transfected (control), or mock-transfected and treated with EGF (30 ng/ml) (+EGF), were fractionated by FPLC on a Mono S column and endogenous MEK activity was measured. Endogenous MAPK eluted in fractions 2 to 4, whereas MEK was contained in fractions 9 to 13. For assaying endogenous MEK activity, cells were washed twice in cold PBS and lysed in 650 µl of a solution containing 50 mM β-glycerophosphate, 10 mM 2-*N*-morpholinoethane-sulfonic acid (pH 6.0), 100 µM sodium vanadate, 2 mM $MgCl_2$, 1 mM EGTA, Triton X-100 (0.5 percent), leupeptin (5 µg/ml), aprotinin (2 µg/ml), and 1 mM dithiothreitol. After centrifugation at maximum speed for 10 minutes in a microfuge, soluble cell lysates (1 to 2 mg of protein) were applied to a Mono S column equilibrated in elution buffer (50 mM β-glycerophosphate, 10 mM MES (pH 6.0), 100 µM sodium vanadate, 2 mM $MgCl_2$, 1 mM EGTA, and 1 mM dithiothreitol. The column was washed with buffer (2 ml) and bound proteins were eluted with a 30ml linear gradient of 0 to 350 mM NaCl in elution buffer. A portion (30 µl) of each fraction was assayed

for MEK activity by mixing with buffer (25 mM β-glycerophosphate, 40 mM *N*-(2-hydroxyethyl)piperazine-N'-(2-ethanolsulfonic acid)(pH 7.2) 50 mM sodium vanadate, 10 mM $MgCl_2$, 100 μM γ-^{32}P-ATP (3000 to 4000 cpm/pmol), inhibitor protein-20 (IP-20; TTYADFIASGRTGRRNAIHD; 25 μg/ml; SEQ ID NO:27), 0.5 mM EGTA, recombinant MAP kinase (7.5 μg/ml), and 200 μM EGFR$^{662-681}$) in a final volume of 40 μl. After incubation at 30 °C for 20 minutes, the incorporation of γ-^{32}P-ATP into EGFR$^{662-681}$ was measured. In this assay, the ability of each column fraction to activate added recombinant MAPK was measured by the incorporation of γ-^{32}P-ATP into the MAPK substrate, a peptide derived from the EGF receptor (EGFR).

Please replace the paragraph beginning at page 98, line 11, with the following paragraph:

p38/Hog-1: Cells were lysed in 1% Triton X-100, 0.5% NP40, 20 mM Tris-HCl, pH 7.5, 150 mM NaCl, 20mM NaF, 0.2 mM sodium vanadate, 1 mM EDTA, 1 mM EGTA, 5 mM phenylmethylsulfonyl fluoride. Nuclei were removed by centrifugation at 15,000 xg for 5 min. Supernatants (200 μg protein) were used for immunoprecipitation of p38/Hog-1 using rabbit antiserum raised against the COOH-terminal peptide sequence of p38/Hog-1 (CFVPPPLDQEEMES; SEQ ID NO:26) (Han, J. et al. (1992) *Mol. Endocrinol.* 6:2079-2089) and protein A Sepharose. Immunoprecipitates were washed 1x in lysis buffer, 1x in assay buffer (25 mM Hepes, pH 7.4, 25 mM β-glycerophosphate, 25 mM $NaCl_2$, 2 mM dithiothreitol, 0.1 mM sodium vanadate), resuspended in kinase assay buffer with 20-50 ng of a recombinant NH_2-terminal fragment of ATF-2 as substrate and 20 μCi [γ32P] ATP (Abdel-Hafig, et al. (1992) *Mol. Endocrinol* 6:2079-2089). For verification of the immunoprecipitation assay lysates were fractionated by Mono Q ion exchange chromatography and each fraction assayed for ATF-2 kinase activity and immunoblotted with anti-p38 antibody. The results demonstrated that p38/Hog-1 containing fractions selectively phosphorylated the recombinant ATF-2 protein.

Please replace the paragraph beginning at page 112, line 28, with the following paragraph:

The sequence IIGQVCDTPKSYDNVHVGLRKV (residues 1306-1327; SEQ ID NO:15) of the MEKK4 sequence) was synthesized as a GST-fusion protein by standard PCR techniques. The GST-fusion peptide bound Cdc42 and Rac in the GTPγS bound form. This fusion protein did not bind Ras using the procedures described above.